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Research paper

Controlled non-invasive iontophoretic delivery of triamcinolone acetonide amino acid ester prodrugs into the posterior segment of the eye

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ABSTRACT

This study investigated short duration transscleral iontophoretic delivery of four triamcinolone acetonide (TA) amino acid ester prodrugs (TA-AA) (alanine, Ala; arginine, Arg; isoleucine, Ile and lysine, Lys) using whole porcine eyes globes *in vitro*. Post-iontophoretic biodistribution of TA was quantified by UHPLC-MS/MS in the different ocular compartments (cornea, aqueous humor, sclera, ciliary body, choroid and retinal pigmented epithelium (RPE), neural retina and vitreous humor). Transscleral iontophoresis (3 mA/cm^2 for 10 min) increased total drug delivery of the TA-AA prodrugs by 14-30-fold as compared to passive diffusion. The TA-AA prodrugs had distinct biodistribution profiles – the penetration depth achieved was dependent on their physicochemical properties (e.g. lipophilicity for TA-Ile) and susceptibility to hydrolysis (e.g. TA-Arg). Intraocular drug distribution was also influenced by prodrug binding to melanin (TA-Lys). Interestingly, under conditions of equivalent charge (6 mA/cm^2 for 5 min vs. 1.5 mA/cm^2 for 20 min, i.e. 1.44 C respectively) the longer duration (20 min) at lower current density resulted in ~6 times more TA delivery into the vitreous humor. Overall, the study provided further evidence of the potential of transscleral iontophoresis for the non-invasive treatment of posterior segment inflammatory diseases.

1. Introduction

Intravitreal injections of corticosteroids such as triamcinolone acetonide (TA) have been routinely employed over the last 15–20 years for the treatment of major inflammatory and angiogenic posterior eye segment diseases such as exudative age related macular degeneration, diabetic macular edema or retinopathy and uveitis [1,2]. The serious consequences of these conditions – ranging from major vision impairment to blindness – and the lack of alternatives accounted for the use of this invasive procedure despite the elevated risk of severe complications and poor patient compliance [3]. Given the complex structure of the eye globe, the organ poses considerable challenges to conventional approaches for topical or systemic drug administration [4]. Topically instilled formulations are promptly diluted by the lacrimal fluid (1.2 µl/min in humans) and drained by the conjunctiva and nasal mucosa, whereas systemic administration is limited by the large doses needed given the efficacy of the blood retinal barrier which isolates the eye from the systemic blood flow [4,5].

Transscleral iontophoresis has been intensively studied as an innovative delivery strategy to overcome the different ocular barriers [6,7]. The sclera is composed mainly of a highly hydrated loose mesh of collagen fibrils and proteoglycans; as a consequence, even the passive permeability of hydrophilic molecules was found to be quite elevated [8,9]. When combined with iontophoresis, molecular transport across the "large" and accessible surface was such that, despite the different ocular barriers, it was possible to achieve therapeutically relevant drug concentrations in the posterior segment [10]. This is important since for effective treatment, therapeutic amounts of drug must cross not only the sclera but also the inner, static ocular barriers such as the choroid and retinal pigmented epithelium in addition to overcoming dynamic barriers such as the blood flow [4].

Iontophoresis, an active drug delivery technique, uses the application of a mild potential to enhance substantially the transport of charged and uncharged molecules into and across biological tissues [11]. Transscleral iontophoretic delivery of dexamethasone phosphate was demonstrated in clinical studies for the treatment of dry eye and noninfectious anterior uveitis using the EyeGate® II applicator (EyeGate Pharmaceuticals Inc) [12,13]. However, drug distribution in the whole eye globe has rarely been studied; although there are empiric observations, reports describing the analytical quantification of drug present in the different compartments are scarce [14].

Therefore, the aim of this study was to investigate the ocular

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biodistribution of biolabile prodrugs and to relate the distribution profile to the molecules' physicochemical properties and susceptibility to enzyme-catalyzed hydrolysis. The iontophoretic delivery of prodrugs, in particular, amino acid prodrugs, has mostly been investigated using the skin [15–19]. There are fewer reports on prodrug iontophoresis in the eye [14]. The prodrug species most frequently encountered in these cases were either succinate [20–22] or phosphate ester [13,23] prodrugs of methylprednisolone or dexamethasone.

We recently described the corneal iontophoresis of novel positively charged amino acid prodrugs of triamcinolone acetonide (TA; TA-AA) [24]. The combination of dramatically increased aqueous solubility and the introduction of ionized groups made these conjugates excellent candidates for iontophoretic delivery. In the present study, four amino acid prodrugs of TA: alanine, arginine, isoleucine and lysine esters (TA-Ala, TA-Arg, TA-Ile and TA-Lys) were synthesized and characterized. Their ocular biodistribution profiles following short duration iontophoresis were determined and correlated to their different physicochemical properties and hydrolytic stabilities. Finally, the effect of the applied current profile was investigated by comparing delivery after iontophoresis of TA-Ala under two different conditions (i) 6 mA/cm^2 for 5 min and (ii) 1.5 mA/cm^2 for 20 min – i.e. an equal amount of total charge was passed, 30 $mA{\cdot}min/cm^2$ (1.44 C), but in the first condition a higher current was applied for a shorter duration whereas in the second a more sustained delivery at a lower current density was employed. In addition to the total amount delivered, the effect on the biodistribution profile was also investigated.

2. Materials and methods

2.1. Materials

TA-AA prodrugs of L-alanine, L-arginine, L-isoleucine and L-lysine (TA-Ala, TA-Arg, TA-Ile and TA-Lys) were synthesized in-house by adapting previously published protocols [18,24]. TA, liquid paraffin and sodium carboxymethylcellulose were purchased from Haenseler AG (Herisau, Switzerland). The buffer salts: sodium and potassium chloride (NaCl, KCl, respectively), sodium and potassium phosphate, 2-morpholino-ethanesulfonic acid monohydrate (MES)) as well as Polysorbate 80 were obtained from Fluka (Buchs, Switzerland). Melanin from *Sepia officinalis* and silver chloride (AgCl) used for the fabrication of the silver electrodes, were acquired from Sigma-Aldrich (Steinheim, Germany). HPLC grade solvents (HiPerSolv Chromatonorm; Darmstadt, Germany) and deionized water were used to prepare all the solutions (Millipore Milli-Q Gard 1 Purification Pack resistivity > 18 MΩcm, Zug, Switzerland). Trifluoroacetic acid (TFA; 99% extra pure) and glycerol were obtained from Acros Organics (Geel, Belgium). ULC/MS grade formic acid was bought from Brunschwig (Basel, Switzerland).

2.2. Analytical methods

2.2.1. HPLC-UV analysis of TA and TA-AA

An UltiMate 3000 HPLC-UV system (formerly Dionex AG, now Thermo Fisher Scientific AG; Reinach Switzerland) equipped with HPG-3200SD pump, WPS-3000 auto sampler and VWD-3400 VIS/UV detector, was used to quantify all molecules. Chromatographic separation was achieved by isocratic elution using water +0.1% TFA (A) and acetonitrile +0.1% TFA (B) as the mobile phase. Compound signal peaks were integrated using Chromeleon® (version 6.8) software. The analytical conditions for the detection and quantification of TA and TA-AA prodrugs are provided in the Supplementary Material. The respective limit of quantification (LOQ) and limit of detection (LOD) were calculated following the ICH Q2 (R1) guideline [25]. The methods were validated and showed suitable intra-day precision and accuracy.

2.2.2. UHPLC MS/MS quantification of TA

A UHPLC-MS/MS method for the quantification of TA in the different ocular tissues was validated adapting a previously published protocol [24]. The Waters Acquity® UPLC® system (Baden-Dättwil, Switzerland) included a binary solvent pump, sample manager and Waters XEVO® TQ-MS detector (Baden-Dättwil, Switzerland). Calibration curves were prepared in matrix over a concentration range of 11.5–2301.5 $pmol_{TA}$/ml and accuracy and precision were evaluated according to the ICH guidelines [25] (Supplementary Material). The LOD and LOQ for TA were 4.1 and 12.4 pmol/ml, respectively.

2.3. Prodrug characterization

Estimates of the pKa and $LogD_{pH5.5}$ of the TA-AA prodrugs were computed by using ACD/Labs software (version 12.01). In addition, a surface conformational analysis of TA and TA-AA prodrugs was performed using the Maestro program, a tool from the Schrödinger 2013 software package (Maestro; Schrödinger, LLC: Portland, OR, 2009). A reference structure of TA was used [ZINC3875481]. Energy minimizations were performed in water on TA and TA-AA prodrugs in charged ($M+H^+$ or $M+2H^+$) and uncharged states using the OPLS-2005 forcefield (10,000 iterations, 0.05 kcal/mol conjugation gradient). Lipophilic potential surface, polar surface area and dipolar moment of TA and TA-AA prodrugs were measured and visualized using Sybyl 2.1.1 (SYBYL-X 2.1.1, Tripos International, St. Louis, Missouri, USA).

2.3.1. Solubility and ester bond stability

Aqueous solubility of the TA-AA prodrugs was established experimentally in conditions similar to the donor compartment composition, e.g. 10 mM MES buffer (pH 5.5). An excess of prodrug was added to the buffer solution and sonicated for 10 min, followed by HPLC-UV analysis of the supernatant.

The stability of the prodrug ester linkage was investigated at 37 °C, at pH 5.5 and at pH 7.4 in contact with freshly isolated ocular tissue (sclera, ciliary body, choroid/retina and vitreous humor). Porcine eye globes were collected promptly after slaughter and the different component tissues were separated and harvested within 4 h of receiving the fresh eye globes. Then, an equal weight of the different tissues (~100 mg) was added to 2 ml PBS solution containing the TA-AA prodrugs (23 μM) under agitation. Samples were withdrawn every 10–30 min and immediately analyzed by HPLC-UV. Based on the slope of the regression curve the first order rate constant of hydrolysis (k_{obs}) was estimated and the half-life ($t_{1/2}$) ($t_{1/2} = \ln 2/k_{obs}$) calculated [18,24]. All experiments were performed in triplicate.

2.3.2. Melanin binding

In vitro studies to investigate binding of TA-AA prodrugs to melanin from *Sepia officinalis* were performed following previously reported protocols [26–28]. 1.5 mg of melanin was weighed and suspended in citrate buffer pH 5 or PBS pH 7.4 under sonication for 10 min; TA-AA prodrug solutions were added consecutively to obtain a total volume of 1.5 ml. TA-AA concentrations between 0.0115 and 0.23 mM for TA-Arg, TA-Ala and TA-Lys and 0.0023–0.023 mM for TA and TA-Ile were tested (in triplicate). The mixture was kept under continuous stirring for 1 h at room temperature. At the end of the incubation period, the supernatant containing unbound TA and TA-AA prodrugs was separated by 15 min centrifugation at 10 000 rpm from the melanin-drug complex and analyzed by HPLC-UV or UHPLC-MS/MS.

2.4. Porcine ocular tissue characterization

Given the lack of dynamic barriers in experimental set-ups used *in vitro*, the choice of an adequate animal model is crucial. In the case of this study, freshly excised porcine eye globes from 6 month old pigs with an average weight of 100–120 kg were selected. The eye globes were provided by the local slaughterhouse (Abattoir de Loëx Sarl; Loëx, Switzerland). Scleral thickness and dimensions of eye bulbs were measured and compared to reported data on porcine, human and rabbit

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